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Secondary Athrocytotic Cardiomyopathy – Heart Damage Due to Wilson's Disease

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Summary. Post-mortem atomic absorption spectrophotometry of the myocardium of a 14-year-old boy revealed a hundred-fold increase in copper. Further electrolyte analysis of the myocardium showed changes corresponding to metabolic heart muscle damage. Ultrastructural examination showed all the feature of a cardiomyopathy at the cellular level. Laser-Microprobe-Mass-Analysis demonstrated an inhomogeneous distribution of copper. An essential factor in the mechanism of death is heart damage.

Key words: Wilson's disease – Cardiomyopathy – Atomic absorption spectrophotometry – Laser-microprobe-mass-analyzer.

Zusammenfassung. Im Myokard eines 14jährigen Jungen mit Morbus Wilson ergab die postmortale Gewebsmineralanalyse eine ca. 100fache Erhöhung des Kupfergehaltes. Das Gesamtgewebsionogramm der Herzmuskulatur entsprach in seiner Konstellation einer Myokardose. Die ultrastrukturellen morphologischen Veränderungen waren für eine Kardiomyopathie charakteristisch. Im Laser-Mikrosonden-Massenanalysator LAMMA 500 fand sich eine inhomogene Kupferverteilung. Der Herzmuskelschaden hat offensichtlich eine wesentliche Beteiligung am Todesmechanismus.

Introduction

The symptoms of the Wilson-Westphal-v. Strümpell-copper-storage-disease are based on the deposits of copper in the organs mainly affected, i.e. liver, kidney and brain. Involvement of other organs with effects on the severity and course of the disease are not reported, in particular involvement of cardiac muscle – which is typical of hemochromatosis – is not described. A slight increase

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of tissue copper up to the six times the normal values is described (Butt et al., 1958; Brandt, 1972, 1975a), but this accumulation does not correlate with any morphological features in the myocardium. Routine examination of the mineral content in several organs revealed values for copper content which were 100fold above the normal range in our patient with known Wilson's disease. In addition we demonstrated the morphological feature of an athrocytotic cardiomyopathy (Brandt, 1976) by light and electron microscopy.

Case Report

The 14-year-old boy developed ascites 3 months ante mortem, and an increasing jaundice of the skin and the conjunctiva 6 months ante mortem. At hospital admission the slim boy had a frog-belly-like enlargement of the abdomen, general jaundice and a tender hepatosplenomegaly. Hepatic foetor, a haemorrhagic diathesis with epistaxis, melena and anasarca developed. Four days before death hypothermia developed with anuria occuring two days after this. Death occured in biventricular but mainly right sided heart failure. One brother is also suffering from Wilson's disease.

ECG (6 Weeks Ante Mortem). Sinus rhythm, incomplete right brunch bundle block, low voltage due pericardial and pleural effusions.

Laboratory Findings. Gamma-glutamyl transpeptidase: 83 U/l (initial) 13 U/l (final). Coagulation: prothrombin time 13% to < 10%; PTT 97.1 s (initial) up to > 150 s (final); coagulation factor II 7%, V 15%, VIII 60%. bilirubin 23 mg%; SGOT 28 U/l; SGPT 31 U/l; cholinesterase 212 U/l; urea-N 12 mg% (initial), 6 mg% (final). copper (serum): 80 µg/100 ml copper (urine/24h): 1,500 µg/24 h (before therapy), 5,000 µg/day (with D-penicillamine administration); ceruloplasmin: 3 mg%.

Pathologic-Anatomic Diagnosis. Distinct granular athrocytotic liver cirrhosis; portal hypertension (sclerosis and ectasia of portal vein; caput Medusae; oesophageal varices; splenomegaly; ascites) haemorrhagic diathesis: subendocardial and subepicardial bleeding, gastric erosions; hepatolenticular degeneration (proliferation of Alzheimer-type II-astrocytes) left ventricular hypertrophy; biventricular heart failure, mainly right sided: pulmonary oedema, marked congestion in the systemic circulation.

Methods

Standardized preparation of the myocardium for electron microscopy: fixation in 2.5% glutaraldehyde and 1% osmium acid, dehydration with acetone and embedding in Epon-812®; the thin sections were detached with lead citrate and saturated uranyl acetate in methyl alcohol and were examined with a Siemens-Elmiskop 101.

Quantitative analysis of electrolytes was performed with an atomic absorption spectrophotometer Beckmann 495 (Brandt, 1975a). A laser-microprobe-mass-analyzer (Lamma 500/Leybold Heraeus) was used for qualitative histotophochemical determination of copper. This modern analyzing system consists of a light microscope, a laser and a time of flight mass-spectrometer (Fig. 1) (Wechsung, 1978). All elements of the periodic table can be determined simultaneously. The detection limits lie within the sub-ppm-range for many elements. The preparation of the specimen is identical with common methods for electron microscopy. Sections were bombarded by laser beam (exposure time some µs). A certain amount of the specimen ($=0.3\,\mu m^3$, about 10^{-13} g organic substance) is thus evaporated and ionised. The plasmic ions are accelerated in an ionic-optical system which follows the specimen-chamber, and are determined by an open secondary electron multiplier.

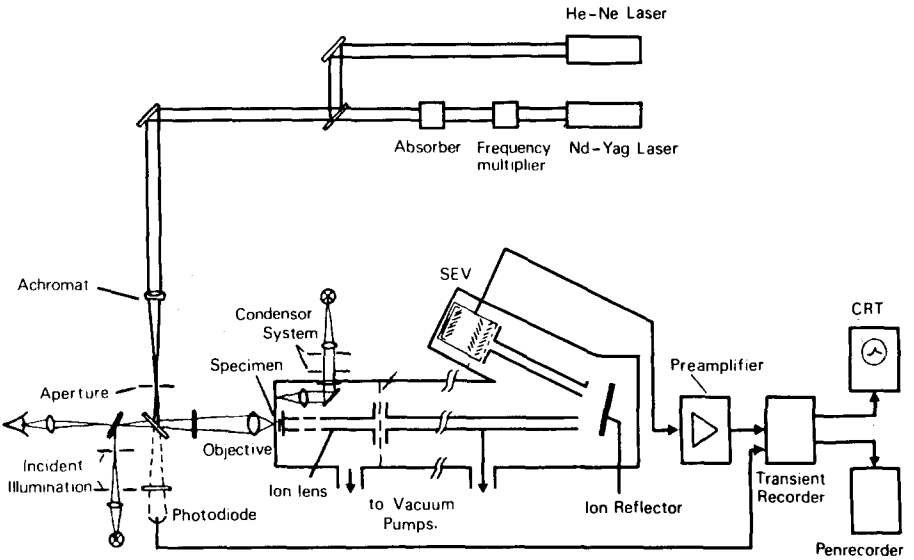

Fig. 1. LAMMA 500-System

Results

Microscopic examination of the cardiac muscle shows distinct variability in size of the myocytes and a focally emphasized proliferation of fibroblasts. There is slight perivascular fibrosis. The semi-thin sections revealed coarse-grained pigmentation within the sarcoplasm (Fig. 2) without any topographic preference, but the myofibrills are defective in particular myocytes. The intrafibrillar spaces formed in this way filled with small vacuoles. The ultrastructural alterations of the cardiac muscle are multiple. The most stricing phenomenon was the increase in mitochondria (Fig. 3). This is the correlation to the vacuoles in the semi-thin sections. The single mitochondrium shows an extensive variation in shape and size (Fig. 4) and normal mitochondria were hardly seen.

There is both a complete loss of the cristae and a marked remodelling: the outer membranes which are preserved, surround partly fine-grained partly coarse deeply osmiophilic dense bodies (Fig. 5). Another type of mitochondrial alteration were the occurence of bizarre myelin figures. They appear both in areas with mitochondrial hyperplasia (Fig. 3) and in simple rows (Fig. 6).

Postmortem Quantitative Determination of Electrolytes

The highest concentration of copper was found in the myocardium: right ventricle 133 mg/100 g dry weight (d.w.), 101.5 mg/100 g d.w. in the left.

The copper concentration of the liver was 51.8 mg/100 g d.w.. Increased concentrations of copper were additionally found in the testes (35.5 mg/100 g d.w.), the kidneys (renal cortex 33 mg/100 g d.w., renal medulla 15.8 mg d.w.)

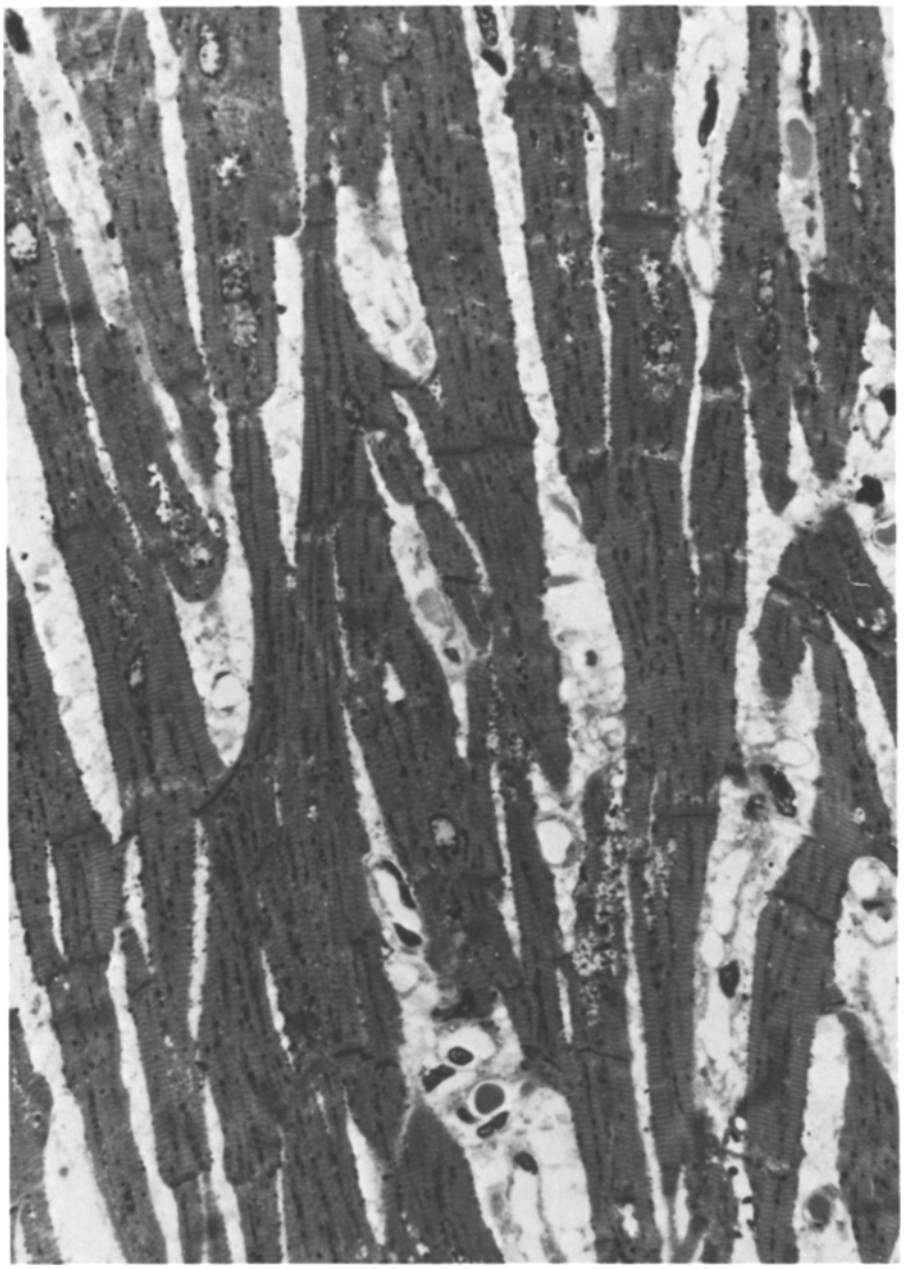

Fig. 2. Rows of granules of dark pigments between the myofibrils; slight interstitial fibrosis. Wilson's disease, glutaraldehyde, osmium acid, semithin section, methyleneblue. Magn. ×150

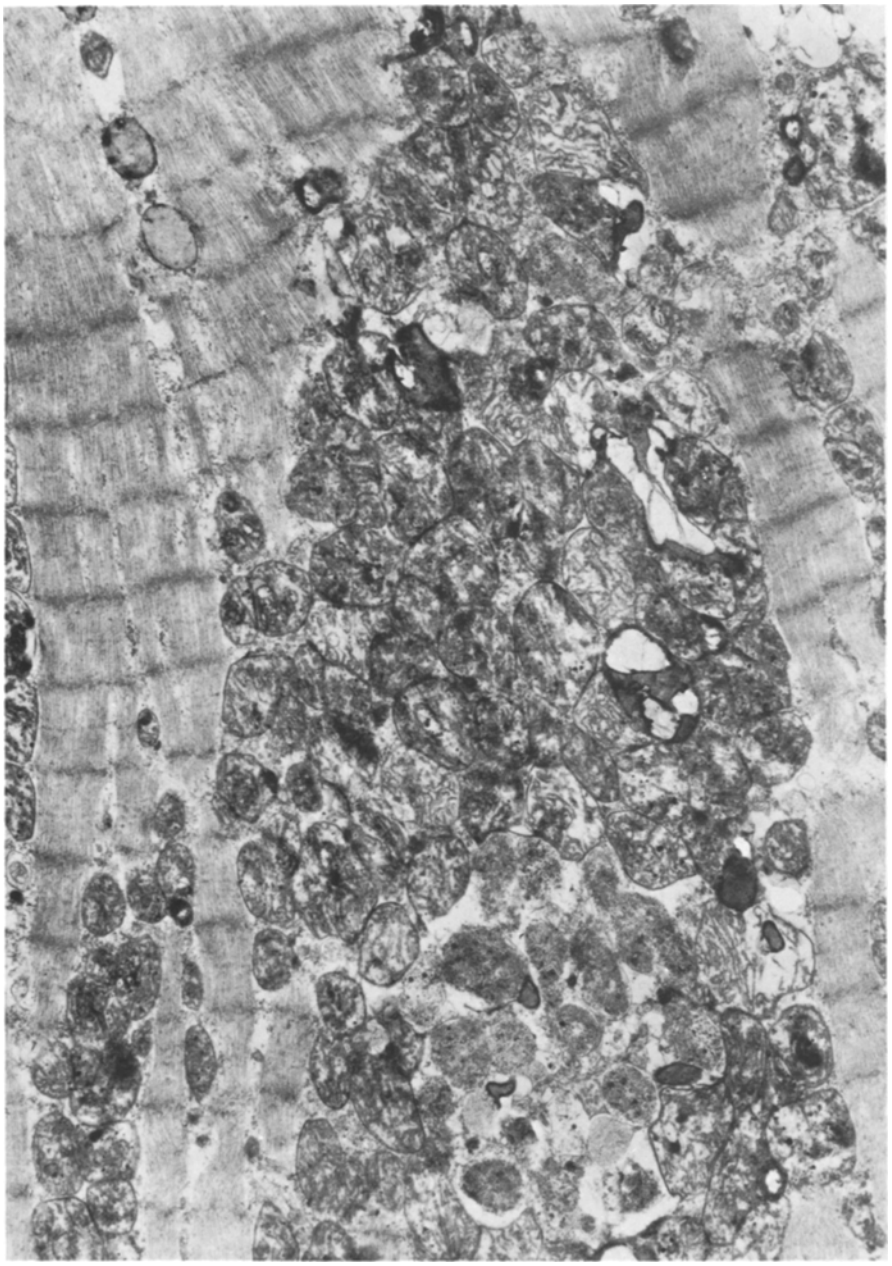

Fig. 3. Marked mitochondriosis of the cardiac muscle in Wilson's disease. 14-year-old boy; glutaraldehyde, osmium acid, Pb-citrate, uranyl-acetate. Magn. ×9,000

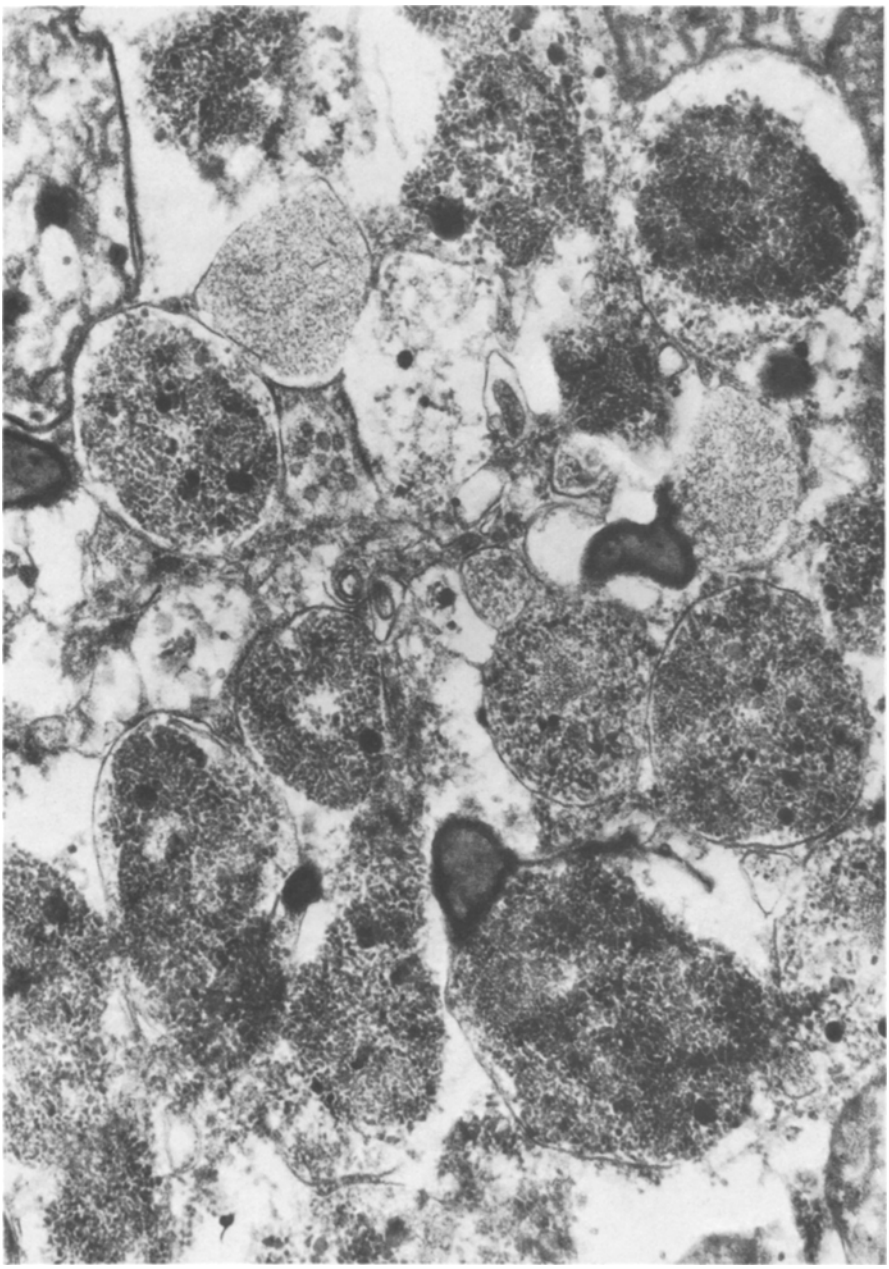

Fig. 4. Multiple variations in size and shape of the mitochondria within an area of hyperplastic mitochondria of the cardiac muscle. Wilson's disease, 14-year-old boy; glutaraldehyde, osmium acid, uranyl-acetate, Pb-citrate. Magn. ×23,000

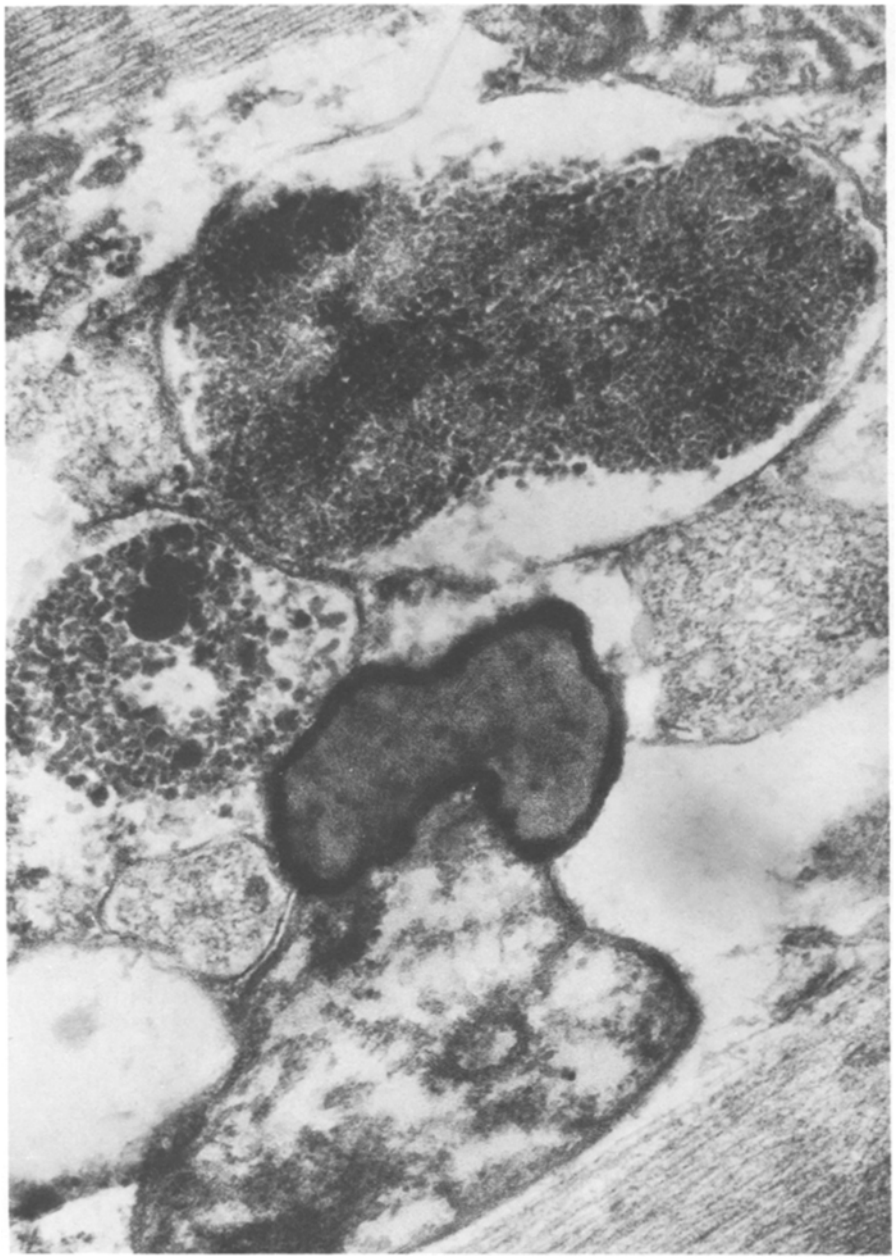

Fig. 5. Advanced degeneration of the mitochondria; presence of finely granular electron dense content, round lipoid droplets and myelin figures. Wilson's disease, myocardium, 14-year-old boy; glutaraldehyde, osmium acid, uranyl-acetate, Pb-citrate. Magn. × 58,000

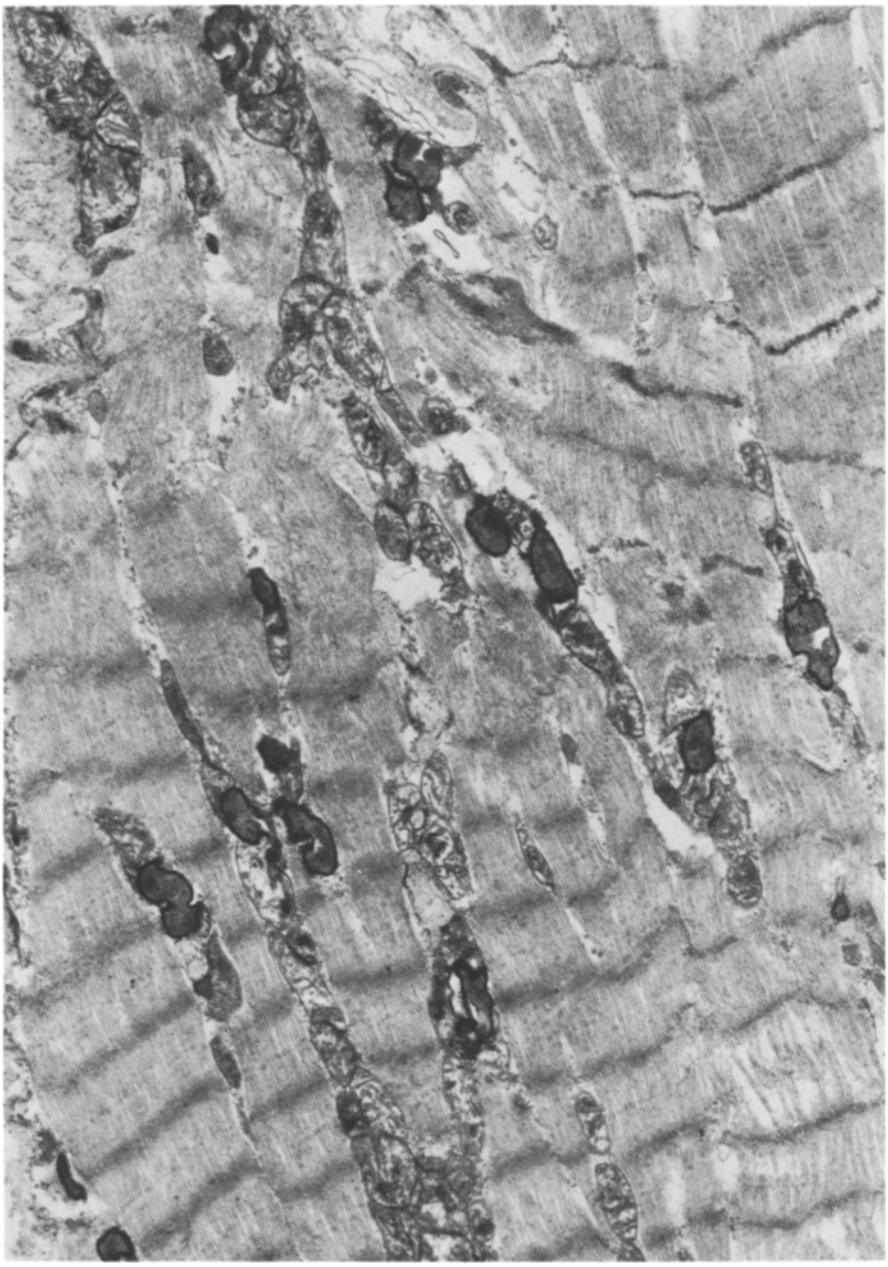

Fig. 6. Severely degenerated mitochondria; mitochondrial cristae are replaced by myelin bodies. Wilson's disease, myocardium, 14-year-old boy; glutaraldehyde, osmium acid, uranyl acetate, Pb-citrate. Magn. ×9,000

Table 1. Water and mineral content of various organs in a case of Wilson's disease with heart damage; (actual value compared with relative value; Brandt, 1975). Na, K, Mg, Ca (mEg/kg dry tissue); Cu, Zn (mg/100 g dry tissue); H_2O (% wet tissue)

	Water	Sodium	Potassium	Magnesium	Calcium	Zinc	Copper
Myocardium, right ventricular	83.31 (80.73±2.1)	623.2 (381.3±83.1)	170.2 (304.4±53)	63.5 (76±10)	29.6 (15±6)	8.9 (10±3)	133.0 (1.3±0.3)
Myocardium, left-ventricular	79.05 (78.75±2.2)	250.6 (245.1±40)	328.7 (376±31)	87.8 (84±8)	11.3 (11±4)	9.0 (10±2)	101.5 (1.3±0.3)
Skeletal muscle	80.40 (76.38±3.3)	240.7 (166±79)	358.6 (381±61)	70.4 (74±11)	4.8 (10±5)	25.6 (24±15)	1.9 (0.4±0.2)
Liver	79.20 (74.78±3.1)	317.5 (218±74)	295.0 (263±41)	50.9 (53±9)	16.1 (9±5)	13.9 (29±19)	51.8 (2±1)
Cerebral medulla	72.1 (71.39±3.9)	211.4 (222±47)	245.7 (226±31)	45.4 (43±4)	7.3 (11±5)	3.7 (3±1)	9.11 (3.0±1)
Cerebral cortex	87.80 (85.48±1.3)	726.7 (622±101)	446.0 (392±69)	80.0 (62±8)	52.2 (42±25)	11.4 (7.4±1.2)	27.3 (2.1±0.5)

Table 2. Postmortem mineral ionogram in a case of Wilson's disease with heart damage. (=) i.e. normal value; (↗) i.e. upper normal value; (↑) i.e. increased; (↙) i.e. lower normal value; (↓) i.e. decreased)

	right ventricle	left ventricle	Skeletal muscle	Liver	Cerebral medulla	Cerebral cortex
Water	↗	=	↗	↗	=	(↗)
Sodium	↑	=	(↗)	↗	=	(↗)
Potassium	↓	↙	=	=	=	=
Magnesium	↙	=	=	=	=	↓
Calcium	↗	=	(↗)	(↗)	=	=
Zinc	=	=	=	=	=	↓
Copper	↑↑	↑↑	↑	↑↑	↑	↑↑

and in different parts of the brain (between 27.3 and 9.1 mg/100 g d.w.). The other organs only showed slight deviations from the normal range (Tables 1 and 2).

Abnormalities of the postmortem quantitative tissue ionogramm were only evident in the right ventricular myocardium, where there was an accumulation of an abnormal amount of water and sodium accompanied by a loss of potassium and magnesium and an increase in calcium.

Detection of Elements with a Laser-Microprobe-Mass-Analyzer (LAMMA 500)

The deposits of pigments found by light microscopy (Fig. 2) revealed two peaks in the positive spectrum: one at m=63 and one at m=65; they represent

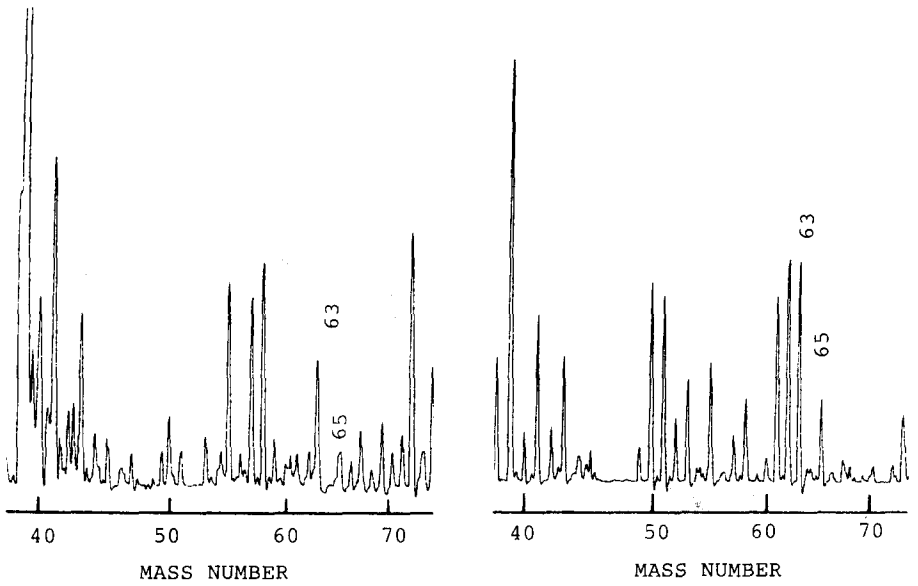

Fig. 7. (*Left*): Mass-spectrum of the darkly pigmented deposits in the right ventricle myocardium. Wilson's disease, 14-year-old boy. (*Right*): Mass spectrum of the Epon-embedded right ventricle myocardium by the side of the pigmental deposits. Wilson's disease, 14-year-old boy

Table 3. Copper content of several organs in a case of Wilson's disease with heart damage (mg Cu/100 g dry tissue)

Myocardium, right-ventricular	133.0
Myocardium, left-ventricular	101.5
Liver	51.8
Testis	35.5
Renal cortex	33.0
Cerebral cortex	27.3
Putamen	25.6
Cerebellar nuclei	19.7
Renal medulla	15.8
Cerebral medulla	9.1
Lung	4.9
Pancreas	2.4
Spleen	2.0
Skeletal muscle	1.9
Bone marrow	1.5

the isotopes of copper (Fig. 7a). It is impossible to demonstrate an increase of copper at a certain cellular location since the analysis of the tissue embedded in Epon-812® showed peaks at m=61, 62, 63, 65 close to the pigmented granules (Fig. 7b). The two peaks (m=63 and 65) cannot be separated clearly and clearly the ammount of copper within the granules lies at the detection limit.

The analysis of the dry tissue, in contrast, revealed that the copper had an inhomogeneous distribution (Fig. 8b).

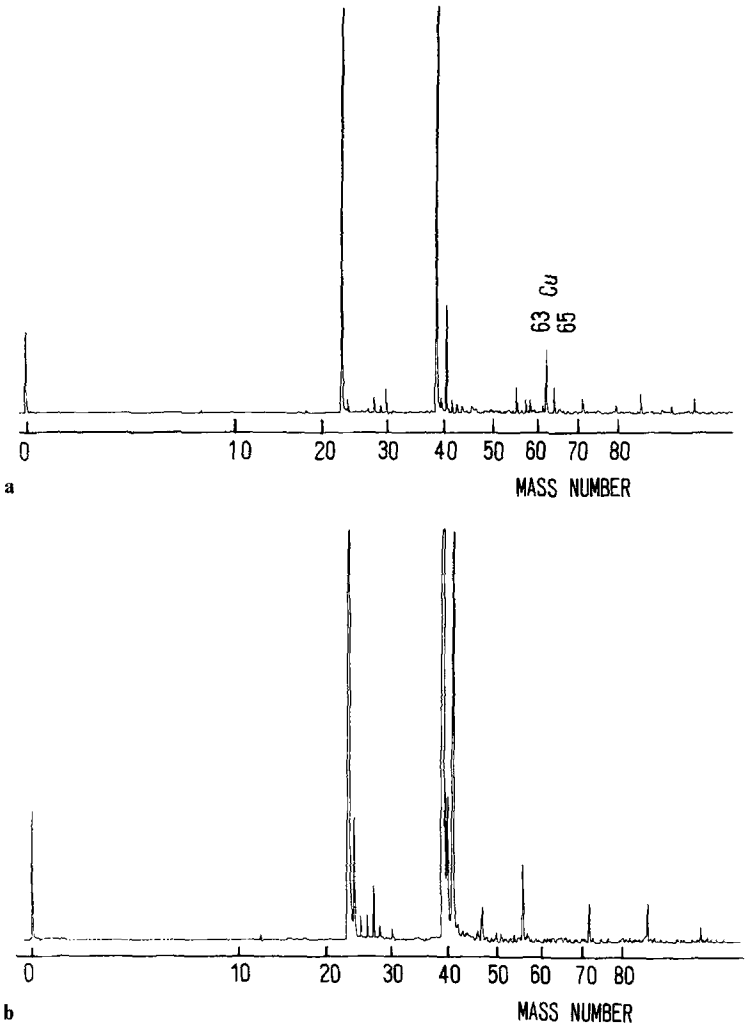

Fig. 8. a Copper determination in the unfixed dry tissue of the right ventricle myocardium. Wilson's disease, 14-year-old boy. **b** Mass-spectrum of the unfixed dry tissue of the right ventricle myocardium. No copper peak. Wilson's disease, 14-year-old boy

Discussion

A slight increase of copper within the cardiac muscle seems to be common in Wilson's disease (Brandt, 1972). Simultaneous structural alterations were not identified in such cases.

Boettiger and Moellerberg (1959) reported a hypertrophic myocardium with a fourfold increase in the copper content in a case of Wilson's disease and described dark granules in the myocytes.

We tested such deposits of brown pigments within the sarcoplasm to determine the elements present by the very sensitive laser-microprobe-mass-analyzer (Lamma 500). This method is a thousand-fold more sensitive than the energy-dispersive X-ray analysis (Edax), We did not succeed in a histotopographic detection of an increased amount of copper within these granules, correlating with the reports of Ritland et al. (1975). They showed by Edax that in Wilson's disease the multivacuolated lysosomes which are regarded as pathognomonic for the condition did not differ in copper content from other varieties of lysosomes within the hepatocytes. Furthermore, these authors showed a uniform elevation of the copper content in all organells (nucleus, mitochondrias, endoplasmatic reticulum, phagolysosomes and hyaloplasm). The discrepancy between the copper concentration measured by atomic absorption spectrophotometry and LAMMA (detection limit for copper: 10^{-18} g) depends on our method. According to measurements by atomic-absorption-spectrophotometry there is a copper content of 133 mg/100 g in *unfixed* dry tissue. Assuming a homogenous distribution of copper one could expect $1.33 \cdot 10^{-15}$ g per μm^3 myocardium, which lies within the detection limit of LAMMA.These differing results can be explained by diffusion of copper ions into aqueous fixative (Brandt, 1974), whereas the light and electron microscopic alterations of the structure remain. These ultrastructural changes of the myocardium correspond to the observation in liver cells by Rossner (1970) and Lough and Wiglesworth (1976): mitochondrial variation in size and shape, accompanied by different stages of degeneration. Mitochondria are known to accumulate large amounts of copper in vivo (Russanov and Balevska, 1966; Russanov, 1970) and in vitro (Russanov, 1970; Cederbaum and Wainio, 1972a; Balevska et al., 1974) and experimental overloading with copper causes a significant reduction of the concentration of the cytochromes in rat liver mitochondria (Balevska et al., 1974). The metabolism of bovine heart mitochondria is similarly influenced by exposure to exogenous copper (Cederbaum and Wainio, 1972b). Thus we conclude, that the mitochondrial alteration we described are due to a myocardial storage of copper. The partly degenerative partly hyperplastic changes of structure resemble those of a primary or a secondary cardiomyopathy (Doerr et al., 1976; Kaduk and Seiler, 1977; Brandt et al., 1978; Kaduk et al., 1978) and therefore we call them "secondary athrocytotic cardiomyopathy" and the similarity to heart damage in hemochromatosis (Doerr, 1951) is obvious, which is called "myocardie" [1] in french and german literature (Doerr, 1970). An exact histotopochemical analysis (Doerr, 1951) could not be performed, because only few particles of the right and left ventricular wall had been asserved.

The shifts of the other minerals are regarded as the typical pattern for postmortem ionograms (Brandt, 1975a) and cannot be interpreted as a specific phenomenon in Wilson's disease. The increased content of water in right ventricle myocardium accompanied by raised sodium and calcium, with decreased potassium and magnesium – and almost unchanged levels of all minerals in the left heart and skeletal muscle – clearly indicates a metabolic right ventricular

[1] "Myocardie" means a metabolic non tumorous and non inflammative damage of myocardium due to dysvitaminosis, dyshormonosis, alcoholism or iron overload.

failure in the sense of "transportative myocardosis"[2] (Brandt, 1976) which is caused by dysproteinaemia due to liver cirrhossis. An important factor in the mechanism of death (Becker et al., 1977) is the severe histological alteration of the heart – compared with other secondary cardiomyopathies. The changes in the myocardial myocytes are not an epiphenomenon without any significance but are a characteristic finding in the widespread changes of Wilson's disease. There is no evidence that the storage of copper in the myocardium was favoured by coincident disease. Thus our case presents an unusual variation in Wilson's disease and we may assume that the individual symptomatological features of Wilson's disease are heterogenous (Aksoy et al., 1975; Passwell et al., 1977).

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[2] "Myocardosis" means a metabolic, non tumorous and non inflammative damage of myocardium due to dysproteinaemia, diabetes mellitus, chronic pancreatitis and other related diseases

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